L1: Entry 1 of 1 File: USPT May 11, 2004

US-PAT-NO: 6734004
DOCUMENT-IDENTIFIER: US 6734004 B2

TITLE: Modified phytases

DATE-ISSUED: May 11, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kostrewa; Dirk	Freiburg			DE
Pasamontes; Luis	Trimbach			CH
Tomschy; Andrea	Grenzach-Wyhlen			DE
van Loon; Adolphus	Rheinfelden			CH
Vogel; Kurt	Basel			CH
Wyss; Markus	Liestal			CH

US-CL-CURRENT: 435/196; 435/252.3, 435/320.1, 435/913, 435/916, 435/917, 536/23.2

CLAIMS:

What is claimed is:

1. A polynucleotide comprising a DNA sequence coding for a modified Aspergillus fumigatus phytase with a specific activity improved over the specific activity of the corresponding unmodified Aspergillus fumigatus phytase wherein the amino acid sequence of the unmodified phytase has been changed at a position corresponding to position 27 of the phytase of Aspergillus niger (SEQ ID NO:1), as identified by PILEUP version 8 amino acid sequence alignment program, to an amino acid selected from the group consisting of Ala, Val, Leu, Ile, Thr, Gly, and Asn.

2. A polynucleotide according to claim 1 wherein the modified Aspergillus fumigatus phytase further comprises an additional mutation selected from the group consisting of S66D, S140Y, D141G, A205E, Q274L, G277D, G277K, Y282H, and N340S.

3. A polynucleotide comprising a DNA sequence coding for a modified Aspergillus fumigatus phytase with a specific activity improved over the specific activity of the corresponding unmodified Aspergillus fumigatus phytase wherein the amino acid sequence of the modified Aspergillus fumigatus phytase has a mutation selected from the group consisting of S66D, S140Y, D141G, A205E, Q274L, G277D, G277K, Y282H, N340S, and combinations thereof, wherein the respective amino acid position of each mutation corresponds to the amino acid position of an Aspergillus niger phytase (SEQ ID NO:1) as identified by PILEUP version 8 amino acid alignment program.

4. A vector comprising the polynucleotide of claim 1.

5. The vector of claim 4 which is an expression vector.

6. A host cell which has been transformed by a polynucleotide of claim 1.

7. A host cell which has been transformed by a vector of claim 4.

8. A polynucleotide according to claim 1 wherein the unmodified phytase has the sequence of SEQ ID NO:3.

9. A polynucleotide according to claim 8 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of Aspergillus niger to the amino acid Ala.

10. A polynucleotide according to claim 8 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of Aspergillus niger to the amino acid Val.

11. A polynucleotide according to claim 8 wherein the amino acid sequence of SEQ ID NQ:3 has been changed at a position corresponding to position 27 of the phytase of Aspergillus niger to the amino acid Leu.

12. A polynucleotide according to claim 8 wherein the amino acid sequence of SEQ ID NQ:3 has been changed at a position corresponding to position 27 of the phytase of Aspergillus niger to the amino acid Ile.

13. A polynucleotide according to claim 8 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of Aspergillus niger to the amino acid Thr.

14. A polynucleotide according to claim 8 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of Aspergillus niger to the amino acid Asn.

15. A polynucleotide according to claim 8 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of Aspergillus niger to the amino acid Gly.

16. A polynucleotide according to claim 8 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: Q23L and S62D.

17. A polynucleotide according to claim 8 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: Q23L, S136Y, and D137G.

18. A polynucleotide according to claim 3 wherein the unmodified phytase has the sequence of SEQ ID NO:3.

19. A polynucleotide according to claim 18 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: S62D.

20. A polynucleotide according to claim 18 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: S136Y.

21. A polynucleotide according to claim 18 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: D137G.

22. A polynucleotide according to claim 18 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: A200E.

23. A polynucleotide according to claim 18 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: Q269L.

24. A polynucleotide according to claim 18 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: G272D.

25. A polynucleotide according to claim 18 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: G272K.

26. A polynucleotide according to claim 18 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: Y277H.

27. A polynucleotide according to claim 18 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: N335S.

28. A vector comprising the polynucleotide of claim 3.

29. The vector of claim 28 which is an expression vector.

30. A host cell which has been transformed by a polynucleotide of claim 3.

31. A host cell which has been transformed by a vector of claim 28.

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L2: Entry 4 of 4 File: USPT May 21, 2002

US-PAT-NO: 6391605
DOCUMENT-IDENTIFIER: US 6391605 B1
**** See image for Certificate of Correction ****

TITLE: Modified phytases

DATE-ISSUED: May 21, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kostrewa; Dirk	Freiburg			DE
Pasamontes; Luis	Trimbach			CH
Tomschy; Andrea	Grenzach-Wyhlen			DE
van Loon; Adolphus	Rheinfelden			CH
Vogel; Kurt	Basel			CH
Wyss; Markus	Liestal			CH

US-CL-CURRENT: 435/196; 424/94.6

CLAIMS:

What is claimed is:

1. A modified Aspergillus fumigatus phytase with a specific activity improved over the specific activity of the corresponding unmodified Aspergillus fumigatus phytase wherein the amino acid sequence of the unmodified phytase has been changed at a position corresponding to position 27 of the phytase of Aspergillus niger (SEQ ID NO:1), as identified by PILEUP version 8 amino acid sequence alignment program, to an amino acid selected from the group consisting of Ala, Val, Leu, Ile, Thr, Gly, and Asn.

2. A modified Aspergillus fumigatus phytase according to claim 1, further comprising an additional mutation selected from the group consisting of S66D, S140Y, D141G, A205E, Q274L, G277D, G277K, Y282H, and N340S.

3. A modified Aspergillus fumigatis phytase with a specific activity improved over the specific activity of the corresponding unmodified Aspergillus fumigatus phytase wherein the amino acid sequence of the modified Aspergillus fumigatus phytase has a mutation selected from the group consisting of S66D, S140Y, D141G, A205E, Q274L, G277D, G277K, Y282H, N340S, and combinations therof, wherein the respective amino acid position of each mutation corresponds to the amino acid position of an Aspergillus niger phytase (SEQ ID NO:1) as identified by PILEUP version 8 amino acid alignment program.

4. A modified phytase according to claim 1 wherein the unmodified phytase has the sequence of SEQ ID NO:3.

5. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of Aspergillus niger to the amino acid Ala.

6. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of Aspergillus niger to the amino acid Val.

7. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of Aspergillus niger to the amino acid Leu.

8. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of Aspergillus niger to the amino acid Ile.

9. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of Aspergillus niger to the amino acid Thr.

10. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of Aspergillus niger to the amino acid Asn.

11. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of Aspergillus niger to the amino acid Gly.

12. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: Q23L and S62D.

13. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: Q23L, S136Y, and D137G.

14. A modified phytase according to claim 3 wherein the unmodified phytase has the sequence of SEQ ID NO:3.

15. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: S62D.

16. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: S136Y.

17. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: D137G.

18. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: A200E.

19. A modified phytase according to claim 3 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: Q269L.

20. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: G272D.

21. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: G272K.

22. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: Y277H.

23. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: N335S.

24. A food or feed composition comprising a modified phytase of claim 1.

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L2: Entry 3 of 4 File: USPT Jun 17, 2003

US-PAT-NO: 6579975
DOCUMENT-IDENTIFIER: US 6579975 B1

TITLE: Consensus phytases

DATE-ISSUED: June 17, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lehmann; Martin	Inzlingen			DE

US-CL-CURRENT: 536/23.2; 435/195, 435/196, 435/252.3, 435/320.1, 435/325, 435/69.1

CLAIMS:

What is claimed is:

1. A polynucleotide encoding a consensus protein of SEQ ID NO:2.

2. A polynucleotide encoding a consensus protein of SEQ ID NO:1.

3. A polynucleotide which encodes a consensus protein having the amino acid sequence of SEQ ID NO:2 except that Q at position 50 has been replaced by L, T, or G.

4. A polynucleotide which encodes a consensus protein having the amino acid sequence of SEQ ID NO:2 except that Q at position 50 has been replaced by T and Y at position 51 has been replaced by N.

5. A polynucleotide which encodes a consensus protein having the amino acid sequence of SEQ ID NO:2 except that Q at position 50 has been replaced by L and Y at position 51 has been replaced by N.

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☐ 1. Document ID: US 20040175757 A1

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L2: Entry 1 of 11 File: PGPB Sep 9, 2004

PGPUB-DOCUMENT-NUMBER: 20040175757
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040175757 A1

TITLE: Low allergenic protein variants

PUBLICATION-DATE: September 9, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Olsen, Arne Agerlin	Virum		DK	
Roggen, Erwin Lugo	Lyngby		DK	
Ernst, Steffen	Kobenhavn N		DK	

US-CL-CURRENT: 435/7.1; 436/518

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWMC | Draw De

☐ 2. Document ID: US 20040142424 A1

L2: Entry 2 of 11 File: PGPB Jul 22, 2004

PGPUB-DOCUMENT-NUMBER: 20040142424
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040142424 A1

TITLE: Modified phytases

PUBLICATION-DATE: July 22, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Kostrewa, Dirk	Freiburg		DE	
Pasamontes, Luis	Trimbach		CH	
Tomschy, Andrea	Grenzach-Wyhlen		DE	
Loon, Adolphus van	Rheinfelden		CH	
Vogel, Kurt	Basle		CH	

Wyss, Markus Liestal CH

US-CL-CURRENT: 435/69.1; 435/196, 435/320.1, 435/325, 435/455, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. De

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☐ 3. Document ID: US 20040126844 A1

L2: Entry 3 of 11 File: PGPB Jul 1, 2004

PGPUB-DOCUMENT-NUMBER: 20040126844
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040126844 A1

TITLE: Using mutations to improve aspergillus phytases

PUBLICATION-DATE: July 1, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Lei, Xingen	Ithaca	NY	US	
Mullaney, Edward J.	New Orleans	LA	US	
Ullah, Abul H.J.	Slidell	LA	US	

US-CL-CURRENT: 435/69.1; 435/196, 435/320.1, 435/419, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. De

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☐ 4. Document ID: US 20030208788 A1

L2: Entry 4 of 11 File: PGPB Nov 6, 2003

PGPUB-DOCUMENT-NUMBER: 20030208788
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030208788 A1

TITLE: Phytase variants

PUBLICATION-DATE: November 6, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Matsui, Tomoko	Chiba		JP	
Fuglsang, Claus Crone	Vekso		DK	
Svendsen, Allan	Horsholm		DK	
Fukuyama, Shiro	Chiba		JP	

US-CL-CURRENT: 800/278; 435/196, 800/8

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 5. Document ID: US 20030190677 A1

L2: Entry 5 of 11 File: PGPB Oct 9, 2003

PGPUB-DOCUMENT-NUMBER: 20030190677
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030190677 A1

TITLE: Consensus phytases

PUBLICATION-DATE: October 9, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Lehmann, Martin	Inzlingen		DE	

US-CL-CURRENT: 435/7.1; 702/19

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 6. Document ID: US 20030119066 A1

L2: Entry 6 of 11 File: PGPB Jun 26, 2003

PGPUB-DOCUMENT-NUMBER: 20030119066
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030119066 A1

TITLE: Diagnostic kit for detecting immunogenic response and method of screening

PUBLICATION-DATE: June 26, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Roggen, Erwin Ludo	Lyngby		DK	
Nilsson, Nina Teeres	Kavlinge		SE	
Ernst, Steffen	Bronshoj		DK	
Patkar, Shamkant Anant	Lyngby		DK	
Friis, Esben Peter			US	

US-CL-CURRENT: 435/7.1; 424/185.1, 435/7.93

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 7. Document ID: US 20030092155 A1

L2: Entry 7 of 11 File: PGPB May 15, 2003

PGPUB-DOCUMENT-NUMBER: 20030092155
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030092155 A1

TITLE: Modified phytases

PUBLICATION-DATE: May 15, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Kostrewa, Dirk	Freiburg		DE	
Pasamontes, Luis	Trimbach		CH	
Tomschy, Andrea	Grenzach-Wyhlen		DE	
Loon, Adolphus van	Rheinfelden		CH	
Vogel, Kurt	Basle		CH	
Wyss, Markus	Liestal		CH	

US-CL-CURRENT: 435/195; 424/94.6, 435/188, 435/196

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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☐ 8. Document ID: US 6734004 B2

L2: Entry 8 of 11 File: USPT May 11, 2004

US-PAT-NO: 6734004
DOCUMENT-IDENTIFIER: US 6734004 B2

TITLE: Modified phytases

DATE-ISSUED: May 11, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kostrewa; Dirk	Freiburg			DE
Pasamontes; Luis	Trimbach			CH
Tomschy; Andrea	Grenzach-Wyhlen			DE
van Loon; Adolphus	Rheinfelden			CH
Vogel; Kurt	Basel			CH
Wyss; Markus	Liestal			CH

US-CL-CURRENT: 435/196; 435/252.3, 435/320.1, 435/913, 435/916, 435/917, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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☐ 9. Document ID: US 6720174 B1

L2: Entry 9 of 11 File: USPT Apr 13, 2004

US-PAT-NO: 6720174
DOCUMENT-IDENTIFIER: US 6720174 B1

TITLE: Phytases

DATE-ISSUED: April 13, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lehmann; Martin	Princeton	NJ		

US-CL-CURRENT: 435/196; 435/18, 435/195, 530/350, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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☐ 10. Document ID: US 6686164 B1

L2: Entry 10 of 11 File: USPT Feb 3, 2004

US-PAT-NO: 6686164
DOCUMENT-IDENTIFIER: US 6686164 B1

TITLE: Low allergenic protein variants

DATE-ISSUED: February 3, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Olsen; Arne Agerlin	Kaplevej			DK
Roggen; Erwin Lugo	Lyngby			DK
Ernst; Steffen	Kobenhavn N			DK

US-CL-CURRENT: 435/7.1; 435/DIG.15, 435/DIG.4, 436/501, 436/513

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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Terms	Documents
3D structure and phytase.clm.	11

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☐	L8	phytase? with 3-D structure	0
☐	L7	modified phytase? with 3-D structure	0
☐	L6	modified phytase? with 3-dimensional structure	0
☐	L5	modified phytase? and 3-dimensional structure	0
☐	L4	3-Dimensional structure and phytase	9
☐	L3	3-Dimensional structure and phytase.clm.	4
☐	L2	3D structure and phytase.clm.	11
☐	L1	3D structure with phytase.clm.	0

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=> s three-dimensional structure and enzyme?
L1       10237 THREE-DIMENSIONAL STRUCTURE AND ENZYME?

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PROCESSING IS APPROXIMATELY  89% COMPLETE FOR L1
PROCESSING COMPLETED FOR L1
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=>        s l2 and phytase?
L3           9 L2 AND PHYTASE?

=> d l3 1-9 ibib ab

L3   ANSWER 1 OF 9     MEDLINE on STN
ACCESSION NUMBER:   2004165683     MEDLINE
DOCUMENT NUMBER:    PubMed ID: 15060628
TITLE:              The role of disulfide bonds in the conformational stability
                    and catalytic activity of phytase.
AUTHOR:             Wang Xiao-Yun; Meng Fan-Guo; Zhou Hai-Meng
CORPORATE SOURCE:   College of Life Science, Shandong Agricultural University,
                    Shandong Tai'an, People's Republic of China.
SOURCE:             Biochemistry and cell biology = Biochimie et biologie
                    cellulaire, (2004 Apr) 82 (2) 329-34.
                    Journal code: 8606068. ISSN: 0829-8211.
PUB. COUNTRY:       Canada
DOCUMENT TYPE:      Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals
ENTRY MONTH:        200411
ENTRY DATE:         Entered STN: 20040403
                    Last Updated on STN: 20041219
                    Entered Medline: 20041124
AB   Previous studies have predicted five disulfide bonds in Aspergillus niger
     phytase (phy A). To investigate the role of disulfide bonds,
     intrinsic fluorescence spectra, far-ultraviolet circular dichroism (CD)
     spectra, and an enzyme activity assay were used to compare the
     differences of catalytic activity and conformational stability of
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phytase during denaturation in urea in the presence and absence of dithiothreitol (DTT). In the presence of 2 mM DTT, the inactivation and unfolding were greatly enhanced at the same concentration of denaturant. The fluorescence emission maximum red shift and decreases of ellipticity at 222 nm were in accord with the changes of catalytic activity. The kinetics of the unfolding courses were a biphasic process consisting of two first-order reactions in the absence of DTT and a monophasic process of a first-order reaction in the presence of DTT. The results suggested that the loss of enzymatic activity was most likely because of a conformational change, and that disulfide bonds played an important role in **three-dimensional structure** and catalytic activity.

L3 ANSWER 2 OF 9 MEDLINE on STN
ACCESSION NUMBER: 2001081457 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10933495
TITLE: Optimization of the catalytic properties of Aspergillus fumigatus **phytase** based on the **three-dimensional structure**.
AUTHOR: Tomschy A; Tessier M; Wyss M; Brugger R; Broger C; Schnoebelen L; van Loon A P; Pasamontes L
CORPORATE SOURCE: F. Hoffmann-La Roche Ltd, Basel, Switzerland.
SOURCE: Protein science : a publication of the Protein Society, (2000 Jul) 9 (7) 1304-11.
Journal code: 9211750. ISSN: 0961-8368.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200101
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010109

AB Previously, we determined the DNA and amino acid sequences as well as biochemical and biophysical properties of a series of fungal **phytases**. The amino acid sequences displayed 49-68% identity between species, and the catalytic properties differed widely in terms of specific activity, substrate specificity, and pH optima. With the ultimate goal to combine the most favorable properties of all **phytases** in a single protein, we attempted, in the present investigation, to increase the specific activity of Aspergillus fumigatus **phytase**. The crystal structure of Aspergillus niger NRRL 3135 **phytase** known at 2.5 A resolution served to specify all active site residues. A multiple amino acid sequence alignment was then used to identify nonconserved active site residues that might correlate with a given favorable property of interest. Using this approach, Gln27 of A. fumigatus **phytase** (amino acid numbering according to A. niger **phytase**) was identified as likely to be involved in substrate binding and/or release and, possibly, to be responsible for the considerably lower specific activity (26.5 vs. 196 U x [mg protein](-1) at pH 5.0) of A. fumigatus **phytase** when compared to Aspergillus terreus **phytase**, which has a Leu at the equivalent position. Site-directed mutagenesis of Gln27 of A. fumigatus **phytase** to Leu in fact increased the specific activity to 92.1 U x (mg protein)(-1), and this and other mutations at position 27 yielded an interesting array of pH activity profiles and substrate specificities. Analysis of computer models of **enzyme**-substrate complexes suggested that Gln27 of wild-type A. fumigatus **phytase** forms a hydrogen bond with the 6-phosphate group of myo-inositol hexakisphosphate, which is weakened or lost with the amino acid substitutions tested. If this hydrogen bond were indeed responsible for the differences in specific activity, this would suggest product release as the rate-limiting step of the A. fumigatus wild-type **phytase** reaction.

L3 ANSWER 3 OF 9 MEDLINE on STN

ACCESSION NUMBER: 1998007872 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9349716
TITLE: Cloning of the **phytases** from Emericella nidulans and the thermophilic fungus Talaromyces thermophilus.
AUTHOR: Pasamontes L; Haiker M; Henriquez-Huecas M; Mitchell D B; van Loon A P
CORPORATE SOURCE: F. Hoffmann-La Roche Ltd., Vitamins and Fine Chemicals Division, Basel, Switzerland.. luis.pasamontes@roche.com
SOURCE: Biochimica et biophysica acta, (1997 Sep 12) 1353 (3) 217-23.
Journal code: 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U59802; GENBANK-U59803
ENTRY MONTH: 199711
ENTRY DATE: Entered STN: 19971224
Last Updated on STN: 19990129
Entered Medline: 19971119

AB **Phytases** (EC 3.1.3.8) belong to the family of histidine acid phosphatases. We have cloned the **phytases** of the fungi Emericella nidulans and Talaromyces thermophilus. The putative **enzyme** encoded by the E. nidulans sequence consists of 463 amino acids and has a Mr of 51785. The protein deduced from the T. thermophilus sequence consists of 466 amino acids corresponding to a Mr of 51450. Both predicted amino acid sequences exhibited high identity (48% to 67%) to known **phytases**. This high level of identity allowed the modelling of all available fungal **phytases** based on the **three-dimensional structure** coordinates of the Aspergillus niger **phytase**. By this approach we identified 21 amino acids which are conserved in fungal phyA **phytases** and are part of the residues forming the substrate pocket. Furthermore, potential glycosylation sites were identified and compared between the aforementioned **phytases** and the A. niger **phytase**.

L3 ANSWER 4 OF 9 MEDLINE on STN
ACCESSION NUMBER: 97032764 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8878514
TITLE: Disulfide bonds are necessary for structure and activity in Aspergillus ficuum **phytase**.
AUTHOR: Ullah A H; Mullaney E J
CORPORATE SOURCE: Southern Regional Research Center, ARS, USDA, New Orleans, Louisiana 70124, USA.
SOURCE: Biochemical and biophysical research communications, (1996 Oct 14) 227 (2) 311-7.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19980206
Entered Medline: 19961203

AB The function of disulfide bonds in Aspergillus ficuum **phytase** was elucidated by unfolding studies, using guanidinium hydrochloride (Gu.HCl) as denaturant. Although the **enzyme** is totally inactivated by 0.8 M Gu.HCl, at pH 5.0, the active conformation is instantaneously restored by 0.6 M Gu.HCl, at pH 5.0. Conditions which would permit refolding of **phytase** are completely negated by 10 mM beta-mercaptoethanol and causes its catalytic demise at pH 7.5. Assay of free thiols using Ellman's reagent indicates that none of the thiols in the ten cysteines in **phytase** are free; five disulfide bonds were predicted for the **enzyme**. Sequence comparison of mold

phytases and yeast acid phosphatases indicates four conserved cysteines. Thus, disulfide bonds play an important role in the folding of fungal **phytase**; any perturbation of the process of its formation causes an altered **three-dimensional structure** that is inconsistent with catalytic activity.

L3 ANSWER 5 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2005:37339 HCAPLUS
TITLE: Structure and function of Escherichia coli glucose-1-phosphatase
AUTHOR(S): Lee, Daniel C.; Jia, Zongchao
CORPORATE SOURCE: Department of Biochemistry, Queen's University, Kingston, ON, K7L 3N6, Can.
SOURCE: Recent Research Developments in Molecular Biology (2003), 1, 251-261
CODEN: RRDMKT
PUBLISHER: Research Signpost
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The E. coli glucose-1-phosphatase, a member of the histidine acid phosphatase family, serves primarily as a glucose scavenger in the bacterial periplasm with high activity for glucose-1-phosphate. The **enzyme** also possesses unique substrate specificities for various inositol phosphates, such as being a 3 and only-3 **phytase**. Detn. of the **three-dimensional structure** of E. coli glucose-1-phosphatase would facilitate the understanding of such unique phytate and glucose-1-phosphate specificities of the **enzyme** . The crystal structure of E. coli glucose-1-phosphatase has been detd. at 2.6-.ANG. resoln. by the method of multi-wavelength anomalous dispersion using a tungstate deriv., together with the complex structure with glucose-1-phosphate and a mutant structure both at 2.4-.ANG. resoln. Lacking substrate-induced conformational change, the active-site pocket of glucose-1-phosphatase is both rigid and small. The presence of two unique gating residues, Glu196 and Leu24, together with the conserved features of histidine acid phosphatase active site, is responsible for the unique substrate selectivity of phytate and glucose-1-phosphate, as well as the unusually high pH range of glucose-1-phosphate activity. Based on the structural characterization, comparison and modeling, simple structural principles were developed that not only accurately predict hydrolysis products of all inositol phosphates and explain substrate specificity of glucose-1-phosphatase, but also rationalize similar general catalytic characteristics across the histidine acid phosphatase family.
REFERENCE COUNT: 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 6 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2001:50782 HCAPLUS
DOCUMENT NUMBER: 134:82721
TITLE: A 2.1 .ANG. crystal structure of a novel thermostable **phytase** from Bacillus amyloliquefaciens
INVENTOR(S): Oh, Tae Kwang; Oh, Byung Chul; Ha, Nam Chul; Choi, Yang Woong; Lee, Dong Kyu; Oh, Byung Ha
PATENT ASSIGNEE(S): Korea Institute of Bioscience and Biotechnology, S. Korea; Daesung Microbiological Labs. Co., Ltd.
SOURCE: PCT Int. Appl., 17 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001004275	A1	20010118	WO 2000-KR2	20000104
W: JP, US				

RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

KR 2001009461	A	20010205	KR 1999-27826	19990709
EP 1194530	A1	20020410	EP 2000-900932	20000104
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2003504047	T2	20030204	JP 2001-509479	20000104
US 2002142433	A1	20021003	US 2002-43623	20020109
US 6808909	B2	20041026		
PRIORITY APPLN. INFO.:			KR 1999-27826 A	19990709
			WO 2000-KR2 W	20000104

AB The present invention relates to a thermostable **phytase** from Bacillus amyloliquefaciens DS-11 with a 2.1 .ANG. crystal structure of a propeller type comprising six external blades that encompass the outer boundary of the crystal and six internal calcium-binding sites that are embedded inside the above crystal. Each of the six blades again consists of 4 or 5 anti-parallel .beta.-strands, and the six calcium binding sites consist of 3 high-affinity calcium binding sites and 3 low-affinity binding sites, which are involved in the **enzyme**'s thermostability and catalytic activity, resp. The above-mentioned Ca2+ binding motifs are expected to be utilized in synthesizing highly thermostable proteins and the elucidation of active sites of an **enzyme** from a **three-dimensional structure** can help to design new **enzymes** having those sites with the aid of recent advanced technol. of protein engineering.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 7 OF 9 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

ACCESSION NUMBER: 2000:187018 SCISEARCH
THE GENUINE ARTICLE: 289EQ
TITLE: From DNA sequence to improved functionality: using protein sequence comparisons to rapidly design a thermostable consensus **phytase**
AUTHOR: Lehmann M (Reprint); Kostrewa D; Wyss M; Brugger R; DArcy A; Pasamontes L; vanLoon A P G M
CORPORATE SOURCE: F HOFFMANN LA ROCHE & CO LTD, GRENZACHERSTR 124, CH-4070 BASEL, SWITZERLAND (Reprint)
COUNTRY OF AUTHOR: SWITZERLAND
SOURCE: PROTEIN ENGINEERING, (JAN 2000) Vol. 13, No. 1, pp. 49-57. Publisher: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND.
ISSN: 0269-2139.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 43

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Naturally-occurring **phytases** having the required level of thermostability for application in animal feeding have not been found in nature thus far. We decided to de novo construct consensus **phytases** using primary protein sequence comparisons. A consensus **enzyme** based on 13 fungal **phytase** sequences had normal catalytic properties, but showed an unexpected 15-22 degrees C increase in unfolding temperature compared with each of its parents. As a first step towards understanding the molecular basis of increased heat resistance, the crystal structure of consensus **phytase** was determined and compared with that of Aspergillus niger **phytase**. Aspergillus niger **phytase** unfolds at much lower temperatures. In most cases, consensus residues were indeed expected, based on comparisons of both three-dimensional structures, to contribute more to **phytase** stabilization than non-consensus amino acids. For some consensus amino acids, predicted by structural comparisons to destabilize the protein, mutational analysis was performed. Interestingly, these consensus residues

in fact increased the unfolding temperature of the consensus **phytase**, In summary, for fungal **phytases** apparently an unexpected direct link between protein sequence conservation and protein stability exists.

L3 ANSWER 8 OF 9 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
ACCESSION NUMBER: 2005:12185 BIOSIS
DOCUMENT NUMBER: PREV200500019770
TITLE: Thermostable **phytase** with 2.1 ANG . . . crystal structure.
AUTHOR(S): Oh, Tae Kwang [Inventor, Reprint Author]; Ha, Nam Chul [Inventor]; Oh, Byung Ha [Inventor]
CORPORATE SOURCE: Daejeon, South Korea
ASSIGNEE: Korea Research Institute of Bioscience and Biotechnology, Daejeon, South Korea; Daesung Microbiological Labs. Co., Kyungki-do, South Korea
PATENT INFORMATION: US 6808909 October 26, 2004
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Oct 26 2004) Vol. 1287, No. 4. http://www.uspto.gov/web/menu/patdata.html. e-file. ISSN: 0098-1133 (ISSN print).
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 22 Dec 2004
Last Updated on STN: 22 Dec 2004

AB The present invention relates to a thermostable **enzyme** with a 2.1 ANG crystal structure of a propeller type comprising six external blades that encompass the outer boundary of the crystal and six internal calcium-binding sites that are embedded inside the above crystal. Each of the six blades comprises 4 or 5 anti-parallel beta-strands, and the six calcium binding sites consist of 3 high-affinity calcium binding sites and 3 low-affinity binding sites, which are involved in the **enzyme**'s thermostability and catalytic activity, respectively. The above-mentioned Ca2+ binding motifs are expected to be utilized in synthesizing highly thermostable proteins and the elucidation of active sites of an **enzyme** from a **three-dimensional structure** can help to design new **enzymes** having those sites with the aid of recent advanced technology of protein engineering.

L3 ANSWER 9 OF 9 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
ACCESSION NUMBER: 2003:438331 BIOSIS
DOCUMENT NUMBER: PREV200300438331
TITLE: Fungal phyA gene expressed in potato leaves produces active and stable **phytase**.
AUTHOR(S): Ullah, Abul H. J. [Reprint Author]; Sethumadhavan, Kandan; Mullaney, Edward J.; Ziegelhoffer, Thomas; Austin-Phillips, Sandra
CORPORATE SOURCE: Southern Regional Research Center, ARS, USDA, 1100 Robert E. Lee Boulevard, New Orleans, LA, 70124, USA aullah@srrc.ars.usda.gov
SOURCE: Biochemical and Biophysical Research Communications, (June 27 2003) Vol. 306, No. 2, pp. 603-609. print. CODEN: BBRCA9. ISSN: 0006-291X.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 24 Sep 2003
Last Updated on STN: 24 Sep 2003

AB Fungal phyA gene from Aspergillus ficuum (niger) was cloned and expressed in potato leaves. The recombinant **enzyme** was stable and catalytically active. The expressed protein in the leaves of the dicotyledonous plant retained most physical and catalytic properties of the benchmark A. ficuum **phytase**. The expressed **enzyme** was, however, 15% less glycosylated than the native **phytase**. The usual bi-hump pH optima profile, which is characteristic of the fungal **phytase**, was altered; however, the pH optimum at 5.0 was unchanged

for phytate and at 4.0 for synthetic substrate p-nitrophenyl phosphate. The temperature was, however, unchanged. The expressed **phytase** was found to be as sensitive as the native **enzyme** to the inhibitory action of pseudo substrate, myo-inositol hexasulfate, while losing about 90% of the activity at 20 muM inhibitor concentration. Similar to the benchmark **phytase**, the expressed **phytase** in leaves was completely inactivated by Arg modifier phenylglyoxal at 60 nM. In addition, the expressed **phytase** in the leaves was inhibited by antibody raised against a 20-mer internal peptide, which is present on the surface of the molecule as shown by the X-ray deduced 3D structure of fungal **phytase**. Taken together, the biochemical evidences indicate that fungal **phytase** when cloned and expressed in potato leaves produces a stable and active biocatalyst. 'Biofarming,' therefore, is an alternative way to produce functional hydrolytic **enzymes** as exemplified by the expression of A. ficuum (niger) phyA gene in potato leaf.

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COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	37.04	37.25

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-1.46	-1.46

STN INTERNATIONAL LOGOFF AT 16:37:20 ON 02 FEB 2005

=> d l1 1-10

L1 ANSWER 1 OF 99 REGISTRY COPYRIGHT 2005 ACS on STN
RN 793185-78-1 REGISTRY
CN **3-Phytase (Idiomarina loihiensis strain L2TR) (9CI)** (CA INDEX NAME)
OTHER NAMES:
CN GenBank AAV80945
CN GenBank AAV80945 (Translated from: GenBank AE017340)
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN
SR GenBank
LC STN Files: CA, CAPLUS
DT.CA CAplus document type: Journal
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 2 OF 99 REGISTRY COPYRIGHT 2005 ACS on STN
RN 746497-03-0 REGISTRY
CN **Phytase (Bacillus licheniformis strain ATCC 14580 gene phy) (9CI)** (CA INDEX NAME)
OTHER NAMES:
CN GenBank AAU22048
CN GenBank AAU22048 (Translated from: GenBank CP000002)
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN
SR GenBank
LC STN Files: CA, CAPLUS
DT.CA CAplus document type: Journal
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 3 OF 99 REGISTRY COPYRIGHT 2005 ACS on STN
RN 736053-29-5 REGISTRY
CN **Histidine acid phosphatase / 6-phytase (Yersinia pseudotuberculosis strain IP32953) (9CI)** (CA INDEX NAME)
OTHER NAMES:
CN GenBank CAH21659
CN GenBank CAH21659 (Translated from: GenBank BX936398)
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN
SR GenBank
LC STN Files: CA, CAPLUS
DT.CA CAplus document type: Journal
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 4 OF 99 REGISTRY COPYRIGHT 2005 ACS on STN
RN 703422-65-5 REGISTRY
CN Phosphatase, phytate (Sartorya fumigata strain ATCC-32239 precursor) (9CI)

(CA INDEX NAME)
OTHER NAMES:
CN **Phytase (Sartorya fumigata strain ATCC-32239 precursor)**
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS
DT.CA CAplus document type: Journal
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 5 OF 99 REGISTRY COPYRIGHT 2005 ACS on STN
RN 703422-64-4 REGISTRY
CN Phosphatase, phytate (Aspergillus fumigatus strain ATCC-58128 precursor) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN **Phytase (Aspergillus fumigatus strain ATCC-58128 precursor)**
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS
DT.CA CAplus document type: Journal
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 6 OF 99 REGISTRY COPYRIGHT 2005 ACS on STN
RN 703422-63-3 REGISTRY
CN Phosphatase, phytate (Aspergillus fumigatus strain ATCC-32722 precursor) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN **Phytase (Aspergillus fumigatus strain ATCC-32722 precursor)**
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS
DT.CA CAplus document type: Journal
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 7 OF 99 REGISTRY COPYRIGHT 2005 ACS on STN
RN 703422-62-2 REGISTRY
CN Phosphatase, phytate (Aspergillus fumigatus strain ATCC-26906 precursor) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN **Phytase (Aspergillus fumigatus strain ATCC-26906 precursor)**
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS

DT.CA CAplus document type: Journal
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
               1 REFERENCES IN FILE CA (1907 TO DATE)
               1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1   ANSWER 8 OF 99 REGISTRY COPYRIGHT 2005 ACS on STN
RN   703422-61-1 REGISTRY
CN   Phosphatase, phytate (Aspergillus fumigatus strain ATCC-36934 precursor)
     (9CI) (CA INDEX NAME)
OTHER NAMES:
CN   **Phytase (Aspergillus fumigatus strain ATCC-34625 precursor)**
FS   PROTEIN SEQUENCE
MF   Unspecified
CI   MAN
SR   CA
LC   STN Files: CA, CAPLUS
DT.CA CAplus document type: Journal
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

RELATED SEQUENCES AVAILABLE WITH SEQLINK

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
               1 REFERENCES IN FILE CA (1907 TO DATE)
               1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1   ANSWER 9 OF 99 REGISTRY COPYRIGHT 2005 ACS on STN
RN   685165-68-8 REGISTRY
CN   **Phosphatase, phytate (synthetic fungi isoenzyme consensus phytase-12
     gene fcp12 precursor) (9CI)** (CA INDEX NAME)
OTHER NAMES:
CN   169: PN: US6599735 SEQID: 169 claimed protein
FS   PROTEIN SEQUENCE
MF   Unspecified
CI   MAN
SR   CA
LC   STN Files: CA, CAPLUS, USPATFULL
DT.CA CAplus document type: Patent
RL.P   Roles from patents: BIOL (Biological study); PREP (Preparation); PRP
       (Properties)

RELATED SEQUENCES AVAILABLE WITH SEQLINK

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
               1 REFERENCES IN FILE CA (1907 TO DATE)
               1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1   ANSWER 10 OF 99 REGISTRY COPYRIGHT 2005 ACS on STN
RN   685165-67-7 REGISTRY
CN   **DNA (synthetic fungi gene fcp7 phytate phosphatase isoenzyme
     consensus phytase-7-specifying) (9CI)** (CA INDEX NAME)
OTHER NAMES:
CN   168: PN: US6599735 SEQID: 168 claimed DNA
FS   NUCLEIC ACID SEQUENCE
MF   Unspecified
CI   MAN
SR   CA
LC   STN Files: CA, CAPLUS, USPATFULL
DT.CA CAplus document type: Patent
RL.P   Roles from patents: BIOL (Biological study); PREP (Preparation); PRP
       (Properties)

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**RELATED SEQUENCES AVAILABLE WITH SEQLINK**

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*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
               1 REFERENCES IN FILE CA (1907 TO DATE)
               1 REFERENCES IN FILE CAPLUS (1907 TO DATE)


=> s (phytase or acid phosphatase)
            99 PHYTASE
       6903830 ACID
         19756 PHOSPHATASE
           559 ACID PHOSPHATASE
                 (ACID(W)PHOSPHATASE)
L2         657 (PHYTASE OR ACID PHOSPHATASE)

=> s phytase and acid phosphatase
            99 PHYTASE
       6903830 ACID
         19756 PHOSPHATASE
           559 ACID PHOSPHATASE
                 (ACID(W)PHOSPHATASE)
L3           1 PHYTASE AND ACID PHOSPHATASE

=> d l3

L3   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2005 ACS on STN
RN   736053-29-5  REGISTRY
CN   Histidine acid phosphatase / 6-phytase (Yersinia pseudotuberculosis
     strain IP32953) (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   GenBank CAH21659
CN   GenBank CAH21659 (Translated from: GenBank BX936398)
FS   PROTEIN SEQUENCE
MF   Unspecified
CI   MAN
SR   GenBank
LC   STN Files:   CA, CAPLUS
DT.CA  CAplus document type:  Journal
RL.NP  Roles from non-patents:  BIOL (Biological study); PRP (Properties)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
               1 REFERENCES IN FILE CA (1907 TO DATE)
               1 REFERENCES IN FILE CAPLUS (1907 TO DATE)


=> s  acid phosphatase
       6903830 ACID
         19756 PHOSPHATASE
L4       559 ACID PHOSPHATASE
               (ACID(W)PHOSPHATASE)

=> d l4 1-5

L4   ANSWER 1 OF 559  REGISTRY  COPYRIGHT 2005 ACS on STN
RN   800285-54-5  REGISTRY
CN   Acid phosphatase SurE (Silicibacter pomeroyi strain DSS-3 gene surE)
     (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   GenBank AAV95933
CN   GenBank AAV95933 (Translated from: GenBank CP000031)
FS   PROTEIN SEQUENCE
MF   Unspecified
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CI MAN
SR GenBank
LC STN Files: CA, CAPLUS
DT.CA CAplus document type: Journal
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
               1 REFERENCES IN FILE CA (1907 TO DATE)
               1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L4 ANSWER 2 OF 559 REGISTRY COPYRIGHT 2005 ACS on STN
RN 793202-58-1 REGISTRY
CN **Type II phosphatidic acid phosphatase (Idiomarina loihiensis strain L2TR) (9CI)** (CA INDEX NAME)
OTHER NAMES:
CN GenBank AAV82625
CN GenBank AAV82625 (Translated from: GenBank AE017340)
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN
SR GenBank
LC STN Files: CA, CAPLUS
DT.CA CAplus document type: Journal
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
               1 REFERENCES IN FILE CA (1907 TO DATE)
               1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L4 ANSWER 3 OF 559 REGISTRY COPYRIGHT 2005 ACS on STN
RN 793201-16-8 REGISTRY
CN **PAP2 (acid phosphatase) superfamily membrane protein (Idiomarina loihiensis strain L2TR) (9CI)** (CA INDEX NAME)
OTHER NAMES:
CN GenBank AAV82483
CN GenBank AAV82483 (Translated from: GenBank AE017340)
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN
SR GenBank
LC STN Files: CA, CAPLUS
DT.CA CAplus document type: Journal
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
               1 REFERENCES IN FILE CA (1907 TO DATE)
               1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L4 ANSWER 4 OF 559 REGISTRY COPYRIGHT 2005 ACS on STN
RN 786740-43-0 REGISTRY
CN **DNA (human clone DE10316701-SEQID-259 gene ACP1 soluble acid phosphatase 1 cDNA plus flanks) (9CI)** (CA INDEX NAME)
OTHER NAMES:
CN 47: PN: DE10316701 PAGE: 1026 claimed DNA
FS NUCLEIC ACID SEQUENCE
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS, TOXCENTER
DT.CA CAplus document type: Patent
RL.P Roles from patents: BIOL (Biological study); PRP (Properties); USES (Uses)

RELATED SEQUENCES AVAILABLE WITH SEQLINK

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L4 ANSWER 5 OF 559 REGISTRY COPYRIGHT 2005 ACS on STN
RN 775172-47-9 REGISTRY
CN **Acid phosphatase SurE (Haloarcula marismortui strain ATCC 43049 gene surE) (9CI)** (CA INDEX NAME)
OTHER NAMES:
CN GenBank AAV45535
CN GenBank AAV45535 (Translated from: GenBank AY596297)
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN
SR GenBank
LC STN Files: CA, CAPLUS
DT.CA CAplus document type: Journal
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

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COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	73.85	74.06

STN INTERNATIONAL LOGOFF AT 11:50:41 ON 03 FEB 2005

=> s (3-dimentional structure? or 3D structure?) phytase and aspergillus) and (mutant? or m
MISSING OPERATOR RUCTURE?) PHYTASE
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s (3-dimentional structure? or 3D structure?) and phytase and aspergillus) and (mutant?
UNMATCHED RIGHT PARENTHESIS 'SPERGILLUS) AND'
The number of right parentheses in a query must be equal to the
number of left parentheses.

=> s (3-dimentional structure? or 3D structure?) and phytase and aspergillus) and (mutant?
UNMATCHED RIGHT PARENTHESIS 'SPERGILLUS) AND'
The number of right parentheses in a query must be equal to the
number of left parentheses.

=> s (3-dimentional structure? or 3D structure?) and (phytase and aspergillus) and (mutant?
L1 1 (3-DIMENTIONAL STRUCTURE? OR 3D STRUCTURE?) AND (PHYTASE AND
ASPERGILLUS) AND (MUTANT? OR MODIFIED OR VARIANT? OR MUTAION?)

=> d l1 ibib ab

L1 ANSWER 1 OF 1 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1999-01149 BIOTECHDS
TITLE: New **modified** protease with increased activity; recombinant enzyme production by vector expression in host cell and application in food industry
AUTHOR: Kostrewa D; Pasamontes L; Tomschy A; van Loon A; Vogel K; Wyss M
PATENT ASSIGNEE: Roche
LOCATION: Basle, Switzerland.
PATENT INFO: AU 9859543 1 Oct 1998
APPLICATION INFO: AU 1998-59543 25 Mar 1998
PRIORITY INFO: EP 1997-810175 25 Mar 1997
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1998-595453 [51]
AB A process for the production of a **modified phytase** with improved activity properties is new and involves: modelling the three-dimensional (**3D**) **structure** of the **phytase** (preferably from **Aspergillus** fumigatus) being **modified**, and optionally that of another **phytase** (preferably from **Aspergillus** terreus or **Aspergillus** niger) with better activity, on the basis of the **3D-structure** of **Aspergillus** niger **phytase**; identifying the amino acids in the active site that differ between the two structures; changing at least one of the differing amino acids in the active site in the **phytase** and incorporating the DNA molecule into an expression vector; and using the DNA or the vector to transform a host cell which is then cultured and isolated. Also claimed are: the **phytase** produced; vectors containing the **modified phytase** DNA; host cells containing the DNA or vector; and feed or foods containing the **modified phytase**. Phytases are useful as feed additives for converting **phytase** to myoinositol and inorganic phosphate. The addition of a **modified phytase** to feed reduces the amount of phytate in animal manure. (118pp)

=> s (3-dimentional structure? or 3D structure?) and phytase and (mutant? or modified or v
L2 1 (3-DIMENTIONAL STRUCTURE? OR 3D STRUCTURE?) AND PHYTASE AND
(MUTANT? OR MODIFIED OR VARIANT? OR MUTAION?)

=> s (3-dimentional structure? or 3D structure?) and phytase and (mutant? or modified or v
L3 2 (3-DIMENTIONAL STRUCTURE? OR 3D STRUCTURE?) AND PHYTASE AND
(MUTANT? OR MODIFIED OR VARIANT? OR MUTATION?)

=> dup rem l3
PROCESSING COMPLETED FOR L3
L4 2 DUP REM L3 (0 DUPLICATES REMOVED)

=> d l4 1-2 ibib ab

L4 ANSWER 1 OF 2 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN
ACCESSION NUMBER: 2000:577138 SCISEARCH
THE GENUINE ARTICLE: 337RU
TITLE: Optimization of the catalytic properties of Aspergillus fumigatus **phytase** based on the three-dimensional structure
AUTHOR: Tomschy A; Tessier M; Wyss M; Brugger R; Broger C; Schnoebelen L; VanLoon A P G M; Pasamontes L (Reprint)
CORPORATE SOURCE: ROCHE VITAMINS INC, BLDG 102, 340 KINGSLAND ST, NUTLEY, NJ 07110 (Reprint); F HOFFMANN LA ROCHE & CO LTD, CH-4072 BASEL, SWITZERLAND
COUNTRY OF AUTHOR: USA; SWITZERLAND
SOURCE: PROTEIN SCIENCE, (JUL 2000) Vol. 9, No. 7, pp. 1304-1311. Publisher: CAMBRIDGE UNIV PRESS, 40 WEST 20TH STREET, NEW YORK, NY 10011-4211. ISSN: 0961-8368.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 16

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Previously, we determined the DNA and amino acid sequences as well as biochemical and biophysical properties of a series of fungal phytases. The amino acid sequences displayed 49-68% identity between species, and the catalytic properties differed widely in terms of specific activity, substrate specificity, and pH optima. With the ultimate goal to combine the most favorable properties of all phytases in a single protein, we attempted, in the present investigation, to increase the specific activity of Aspergillus fumigatus **phytase**. The crystal structure of Aspergillus niger NRRL 3135 **phytase** known at 2.5 Angstrom resolution served to specify all active site residues. A multiple amino acid sequence alignment was then used to identify nonconserved active site residues that might correlate with a given favorable property of interest. Using this approach, Gln27 of A. fumigatus **phytase** (amino acid numbering according to A. niger **phytase**) was identified as likely to be involved in substrate binding and/or release and, possibly, to be responsible for the considerably lower specific activity (26.5 vs. 196 U . [mg protein](-1) at pH 5.0) of A. fumigatus **phytase** when compared to Aspergillus terreus **phytase**, which has a Leu at the equivalent position. Site-directed mutagenesis of Gln27 of A. fumigatus **phytase** to Leu in fact increased the specific activity to 92.1 U . (mg protein)(-1), and this and other **mutations** at position 27 yielded an interesting array of pH activity profiles and substrate specificities. Analysis of computer models of enzyme-substrate complexes suggested that Gln27 of wild-type A. fumigatus **phytase** forms a hydrogen bond with the 6-phosphate group of myo-inositol hexakisphosphate, which is weakened or lost with the amino acid substitutions tested. If this hydrogen bond were indeed responsible for the differences in specific activity, this would suggest product release as the rate-limiting step of the A. fumigatus wild-type **phytase** reaction.

L4 ANSWER 2 OF 2 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1999-01149 BIOTECHDS
TITLE: New **modified** protease with increased activity; recombinant enzyme production by vector expression in host cell and application in food industry
AUTHOR: Kostrewa D; Pasamontes L; Tomschy A; van Loon A; Vogel K;

Wyss M
PATENT ASSIGNEE: Roche
LOCATION: Basle, Switzerland.
PATENT INFO: AU 9859543 1 Oct 1998
APPLICATION INFO: AU 1998-59543 25 Mar 1998
PRIORITY INFO: EP 1997-810175 25 Mar 1997
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1998-595453 [51]

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=> d his

(FILE 'HOME' ENTERED AT 14:46:21 ON 02 FEB 2005)

FILE 'MEDLINE, HCAPLUS, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH' ENTERED AT 14:47:26 ON 02 FEB 2005

L1 1 S (3-DIMENTIONAL STRUCTURE? OR 3D STRUCTURE?) AND (PHYTASE AND
L2 1 S (3-DIMENTIONAL STRUCTURE? OR 3D STRUCTURE?) AND PHYTASE AND
L3 2 S (3-DIMENTIONAL STRUCTURE? OR 3D STRUCTURE?) AND PHYTASE AND
L4 2 DUP REM L3 (0 DUPLICATES REMOVED)

=> log y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	40.29	40.71

STN INTERNATIONAL LOGOFF AT 14:59:41 ON 02 FEB 2005

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=> file medline hcaplus embase biosis biotechds scisearch
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       0.21       0.21

FILE 'MEDLINE' ENTERED AT 15:00:42 ON 02 FEB 2005

FILE 'HCAPLUS' ENTERED AT 15:00:42 ON 02 FEB 2005
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COPYRIGHT (C) 2005 THE THOMSON CORPORATION

FILE 'SCISEARCH' ENTERED AT 15:00:42 ON 02 FEB 2005
Copyright (c) 2005 The Thomson Corporation.

=> s (3D structure or 3 dimensional structure) and Aspergillus phytase?
L1            1 (3D STRUCTURE OR 3 DIMENSIONAL STRUCTURE) AND ASPERGILLUS PHYTAS
                E?

=> s (3D structure or 3 dimensional structure) and phytase?
L2           14 (3D STRUCTURE OR 3 DIMENSIONAL STRUCTURE) AND PHYTASE?

=> dup rem l2
PROCESSING COMPLETED FOR L2
L3             9 DUP REM L2 (5 DUPLICATES REMOVED)

=> d l3 1-9 ibib ab

L3   ANSWER 1 OF 9  SCISEARCH  COPYRIGHT (c) 2005 The Thomson Corporation.  on
     STN
ACCESSION NUMBER:    2004:869668  SCISEARCH
THE GENUINE ARTICLE: 855JN
TITLE:               Crystallographic snapshots of Aspergillus fumigatus
                     phytase, revealing its enzymatic dynamics
AUTHOR:              Liu O; Huang Q Q; Lei X G; Hao Q (Reprint)
CORPORATE SOURCE:    Cornell Univ, Dept Mol Biol & Genet, Ithaca, NY 14853 USA
                     (Reprint); Cornell Univ, MacCHESS, Ithaca, NY 14853 USA;
                     Cornell Univ, Dept Anim Sci, Ithaca, NY 14853 USA
COUNTRY OF AUTHOR:   USA
SOURCE:              STRUCTURE, (SEP 2004) Vol. 12, No. 9, pp. 1575-1583.
                     Publisher: CELL PRESS, 1100 MASSACHUSETTS AVE, CAMBRIDGE,
                     MA 02138 USA.
                     ISSN: 0969-2126.
DOCUMENT TYPE:       Article; Journal
LANGUAGE:            English
REFERENCE COUNT:     30
                    *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
AB       Understanding of the atomic movements involved in an enzymatic reaction
     needs structural information on the active and inactive native enzyme
     molecules and on the enzyme-substrate, enzyme-intermediate, and
     enzyme-product(s) complexes. By using the X-ray crystallographic method,
     four crystal structures of Aspergillus fumigatus phytase were
     obtained at resolution higher than 1.7 Angstrom. The pH-dependent
     catalytic activity of A. fumigatus phytase was linked to three
     water molecules that may prevent the substrate from binding and thus block
     nucleophilic attack of the catalytic imidazole nitrogen. Comparison of
```

various structures also identified the water molecule that attacks the phosphamide bond during the hydrolysis process, and established the hydrolysis pathway of the intermediate. Additionally, two reaction product phosphates were observed at the active site, suggesting a possible product release pathway after hydrolysis of the intermediate. These results can help explain the catalytic mechanism throughout the whole acid phosphatase family, as all key residues are conserved.

L3 ANSWER 2 OF 9 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2003305590 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12804608
TITLE: Fungal phyA gene expressed in potato leaves produces active and stable **phytase**.
AUTHOR: Ullah Abul H J; Sethumadhavan Kandan; Mullaney Edward J; Ziegelhoffer Thomas; Austin-Phillips Sandra
CORPORATE SOURCE: Southern Regional Research Center, ARS, USDA, 1100 Robert E. Lee Boulevard, New Orleans, LA 70124, USA.. aullah@srrc.ars.usda.gov
SOURCE: Biochemical and biophysical research communications, (2003 Jun 27) 306 (2) 603-9.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200307
ENTRY DATE: Entered STN: 20030702
Last Updated on STN: 20030726
Entered Medline: 20030725

AB Fungal phyA gene from Aspergillus ficuum (niger) was cloned and expressed in potato leaves. The recombinant enzyme was stable and catalytically active. The expressed protein in the leaves of the dicotyledonous plant retained most physical and catalytic properties of the benchmark A. ficuum **phytase**. The expressed enzyme was, however, 15% less glycosylated than the native **phytase**. The usual bi-hump pH optima profile, which is characteristic of the fungal **phytase**, was altered; however, the pH optimum at 5.0 was unchanged for phytate and at 4.0 for synthetic substrate p-nitrophenyl phosphate. The temperature was, however, unchanged. The expressed **phytase** was found to be as sensitive as the native enzyme to the inhibitory action of pseudo substrate, myo-inositol hexasulfate, while losing about 90% of the activity at 20 microM inhibitor concentration. Similar to the benchmark **phytase**, the expressed **phytase** in leaves was completely inactivated by Arg modifier phenylglyoxal at 60 nM. In addition, the expressed **phytase** in the leaves was inhibited by antibody raised against a 20-mer internal peptide, which is present on the surface of the molecule as shown by the X-ray deduced **3D structure** of fungal **phytase**. Taken together, the biochemical evidences indicate that fungal **phytase** when cloned and expressed in potato leaves produces a stable and active biocatalyst. 'Biofarming,' therefore, is an alternative way to produce functional hydrolytic enzymes as exemplified by the expression of A. ficuum (niger) phyA gene in potato leaf.

L3 ANSWER 3 OF 9 EMBASE
on STN
ACCESSION NUMBER: 2003080758 EMBASE
TITLE: Beta-propellers: Associated functions and their role in human diseases.
AUTHOR: Pons T.; Gomez R.; Chinea G.; Valencia A.
CORPORATE SOURCE: T. Pons, Ctr. Ingenieria Genet. Biotecnologia, P.O. Box 6162, Havana 10600, Cuba. tirso.pons@cigb.edu.cu
SOURCE: Current Medicinal Chemistry, (2003) 10/6 (505-524).
Refs: 239
ISSN: 0929-8673 CODEN: CMCHE7

COUNTRY: Netherlands
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 005 General Pathology and Pathological Anatomy
029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The .beta.-propeller fold appears as a very fascinating architecture based on four-stranded antiparallel and twisted .beta.-sheets, radially arranged around a central tunnel. Similar to the .alpha./.beta.-barrel (TIM-barrel) fold, the .beta.-propeller has a wide range of different functions, and is gaining substantial attention. Some proteins containing .beta.-propeller domains have been implicated in the pathogenesis of a variety of diseases such as cancer, Alzheimer, Huntington, arthritis, familial hypercholesterolemia, retinitis pigmentosa, osteogenesis, hypertension, and microbial and viral infections. This article reviews some aspects of **3D structure**, amino acids sequence regularities, and biological functions of the proteins containing .beta.-propeller domains. Major emphasis has been laid on .beta.-propellers whose functions are associated to human diseases. Recent research efforts reported in the fields of protein engineering, drug design, and protein structure-function relationship studies, concerning the .beta.-propeller architecture, have also been discussed.

L3 ANSWER 4 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2001:816739 HCAPLUS
DOCUMENT NUMBER: 135:356734
TITLE: Protein engineering for reduced allergenicity
INVENTOR(S): Roggen, Erwin Ludo; Ernst, Steffen; Svendsen, Allan; Friis, Esben Peter; Von Der Osten, Claus
PATENT ASSIGNEE(S): Novozymes A/S, Den.
SOURCE: PCT Int. Appl., 513 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001083559	A2	20011108	WO 2001-DK293	20010430
WO 2001083559	A3	20020620		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2406621	AA	20011108	CA 2001-2406621	20010430
EP 1280817	A2	20030205	EP 2001-927643	20010430
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				

PRIORITY APPLN. INFO.:

DK 2000-707	A	20000428
US 2000-203345P	P	20000510
DK 2001-327	A	20010228
US 2001-277817P	P	20010321
WO 2001-DK293	W	20010430

AB The authors disclose a method of selecting a protein variant having modified immunogenicity as compared to the parent protein. The method comprises (1) obtaining antibody-binding peptide sequences; (2) using the sequences to localize epitopes on the **3-dimensional**

structure of parent protein; (3) defining an epitope area including amino acids situated within 5.ANG.; (4) changing one or more of the epitope amino acids by genetic engineering of mutations in the DNA sequence encoding the parent protein, and (5) expressing the engineered protein variant and evaluating the immunogenicity. In one example, linear and discontinuous epitopes of savinase were mapped, mutants engineered, and their immunogenicity examd. In a second example, epitopes of environmental allergens (e.g., dust mite, pollen, venom) and com. enzymes (e.g., subtilisin, carezyme, laccase, amylase) were mapped.

L3 ANSWER 5 OF 9 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2001-06291 BIOTECHDS
TITLE: Thermostable **phytase** enzyme having a 2.1 Angstroms crystal structure having a propeller shape, useful in feed production for degrading phytate regardless of the position of its phosphate groups;
for use in protein engineering and phytate degradation
AUTHOR: Oh T K; Oh B C; Ha N C; Choi Y W; Lee D K; Oh B H
PATENT ASSIGNEE: Korea-Res.Inst.Biosci.Biotechnol.; Daesung-Microbiol.Lab.
LOCATION: Daejon, Korea; Kyungki-do, Korea.
PATENT INFO: WO 2001004275 18 Jan 2001
APPLICATION INFO: WO 2000-KR2 4 Jan 2000
PRIORITY INFO: KR 1999-27826 9 Jul 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-138336 [14]
AB A thermostable **phytase** derived from Bacillus amyloliquefaciens DS-11 with a 2.1 A crystal structure of a propeller shape comprising 6 eternal blades each consisting of 4 or 5 anti-parallel beta-strands, is claimed. The calcium binding sites consist of 3 high-affinity calcium binding sites and 3 low-affinity calcium binding sites located inside the 6 blades. The calcium ion binding motifs of the enzyme are useful in synthesizing highly thermostable proteins. Elucidation of active sites of the enzyme from a **3D structure** can help design new enzymes having those sites with the aid of recent advanced technology of protein engineering. The **phytase** is useful in feed production for degrading phytate regardless of the position of its phosphate groups. The **phytase** can degrade phytate regardless of the position of phosphate groups in a phytate during feed production and the enzyme activity can be maintained at a high temp. The enzyme has 6 blades in the form of a propeller with the fourth strand connected to the fifth blade. (17pp)

L3 ANSWER 6 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2000:314711 HCAPLUS
DOCUMENT NUMBER: 132:333397
TITLE: Engineering of enzymes for reduced allergenicity
INVENTOR(S): Roggen, Erwin Ludo; Olsen, Arne Agerlin; Ernst, Steffen
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 91 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000026230	A1	20000511	WO 1999-DK541	19991012

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ,

BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

CA 2348938	AA	20000511	CA 1999-2348938	19991012
AU 9960787	A1	20000522	AU 1999-60787	19991012
AU 776534	B2	20040916		
EP 1124843	A1	20010822	EP 1999-947260	19991012
EP 1124843	B1	20040407		

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

JP 2003500003	T2	20030107	JP 2000-579618	19991012
EP 1277762	A2	20030122	EP 2002-20745	19991012
EP 1277762	A3	20030409		

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI, CY

NZ 511291	A	20030829	NZ 1999-511291	19991012
AT 263778	E	20040415	AT 1999-947260	19991012
US 6686164	B1	20040203	US 1999-417608	19991013
US 2004175757	A1	20040909	US 2003-730454	20031208

PRIORITY APPLN. INFO.:	DK 1998-1402	A 19981030
	DK 1998-1645	A 19981125
	DK 1999-1417	A 19991004
	US 1998-107165P	P 19981105
	US 1998-111386P	P 19981208
	US 1999-157429P	P 19991004
	EP 1999-947260	A3 19991012
	WO 1999-DK541	W 19991012
	US 1999-417608	A1 19991013

AB The authors disclose methodol. for selecting a protein variant having reduced immunogenicity as compared to the parent protein. The methodol. comprises screening a random peptide display library with antibodies raised against the protein of interest, sequencing the peptides, or the DNA sequence encoding the peptides, and identifying epitope patterns by sequence alignment. The epitope patterns are then located on the **3-dimensional structure** of the parent protein and are used to define an area including amino acids situated within 5 .ANG. from the linear epitope amino acids. By genetic engineering mutations of the DNA sequence encoding the parent protein without impairing functionality of the protein are constructed and evaluated for reduced immunogenicity. In one example, the authors generated the enzyme Savinase with an R170F mutation. In competitive IgE ELISA, this variant was less effective in competing for anti-Savinase antibodies. In addn., the Savinase variant exhibited an 80% redn. in the prodn. of specific IgE in mice.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 7 OF 9 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

ACCESSION NUMBER: 2000:577138 SCISEARCH
THE GENUINE ARTICLE: 337RU
TITLE: Optimization of the catalytic properties of Aspergillus fumigatus **phytase** based on the three-dimensional structure
AUTHOR: Tomschy A; Tessier M; Wyss M; Brugger R; Broger C; Schnoebelen L; VanLoon A P G M; Pasamontes L (Reprint)
CORPORATE SOURCE: ROCHE VITAMINS INC, BLDG 102, 340 KINGSLAND ST, NUTLEY, NJ 07110 (Reprint); F HOFFMANN LA ROCHE & CO LTD, CH-4072 BASEL, SWITZERLAND
COUNTRY OF AUTHOR: USA; SWITZERLAND
SOURCE: PROTEIN SCIENCE, (JUL 2000) Vol. 9, No. 7, pp. 1304-1311. Publisher: CAMBRIDGE UNIV PRESS, 40 WEST 20TH STREET, NEW YORK, NY 10011-4211.
ISSN: 0961-8368.

DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 16

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

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L3 ANSWER 8 OF 9 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1999-01149 BIOTECHDS
TITLE: New modified protease with increased activity; recombinant enzyme production by vector expression in host cell and application in food industry
AUTHOR: Kostrewa D; Pasamontes L; Tomschy A; van Loon A; Vogel K; Wyss M
PATENT ASSIGNEE: Roche
LOCATION: Basle, Switzerland.
PATENT INFO: AU 9859543 1 Oct 1998
APPLICATION INFO: AU 1998-59543 25 Mar 1998
PRIORITY INFO: EP 1997-810175 25 Mar 1997
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1998-595453 [51]

AB A process for the production of a modified **phytase** with improved activity properties is new and involves: modelling the three-dimensional (**3D**) **structure** of the **phytase** (preferably from Aspergillus fumigatus) being modified, and optionally that of another **phytase** (preferably from Aspergillus terreus or Aspergillus niger) with better activity, on the basis of the **3D-structure** of Aspergillus niger **phytase**; identifying the amino acids in the active site that differ between the two structures; changing at least one of the differing amino acids in the active site in the **phytase** and incorporating the DNA molecule into an expression vector; and using the DNA or the vector to transform a host cell which is then cultured and isolated. Also claimed are: the **phytase** produced; vectors containing the modified **phytase** DNA; host cells containing the DNA or vector;

and feed or foods containing the modified **phytase**. **Phytases** are useful as feed additives for converting **phytase** to myoinositol and inorganic phosphate. The addition of a modified **phytase** to feed reduces the amount of phytate in animal manure. (118pp)

L3 ANSWER 9 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1996:626316 HCAPLUS
DOCUMENT NUMBER: 125:269217
TITLE: Disulfide bonds are necessary for structure and activity in Aspergillus ficuum **phytase**
AUTHOR(S): Ullah, Abul H. J.; Mullaney, Edward J.; Mullaney, Edward J.
CORPORATE SOURCE: Southern Regional Research Center, ARS, USDA, New Orleans, LA, 70124, USA
SOURCE: Biochemical and Biophysical Research Communications (1996), 227(2), 311-317
CODEN: BBRCA9; ISSN: 0006-291X
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The function of disulfide bonds in A. ficuum **phytase** (I) was elucidated by unfolding studies, using guanidine-HCl (GuHCl) as denaturant. Although I was totally inactivated by 0.8M GuHCl, at pH 5.0, the active conformation was instantaneously restored by 0.6M GuHCl at pH 5.0. Conditions which would permit refolding of I were completely negated by 10 mM .beta.-mercaptoethanol and caused its catalytic demise at pH 7.5. Assay of free SH groups using Ellman's reagent indicated that none of the SH groups in the 10 Cys residues in I were free; 5 disulfide bonds were predicted for the enzyme. Sequence comparison of fungal I and yeast acid phosphatases indicated 4 conserved Cys residues. Thus, disulfide bonds play an important role in the folding of fungal I; any perturbation of the process of its formation causes an altered **3-dimensional structure** that is inconsistent with catalytic activity.

=> d his

(FILE 'HOME' ENTERED AT 15:00:06 ON 02 FEB 2005)

FILE 'MEDLINE, HCAPLUS, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH' ENTERED AT 15:00:42 ON 02 FEB 2005
L1 1 S (3D STRUCTURE OR 3 DIMENSIONAL STRUCTURE) AND ASPERGILLUS PHY
L2 14 S (3D STRUCTURE OR 3 DIMENSIONAL STRUCTURE) AND PHYTASE?
L3 9 DUP REM L2 (5 DUPLICATES REMOVED)

=> log y

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